Medical Ultrasonography
2011, Vol. 13, no. 3, 207-214

The role of color histograms in predicting the prognosis of patients with digestive tract adenocarcinoma

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Abstract

Objectives: To establish the correlation between the degree of vascularisation detected using power Doppler ultrasonography in digestive tract adenocarcinoma and the prognosis of these patients. **Material and method:** Ultrasonography was performed in 45 patients diagnosed with digestive tract adenocarcinoma (16 stomach-35.6%, 6 cecum and ascending colon-13.3%, 2 transverse colon-4.4%, 5 descending colon 11.1%, 13 sigmoid colon-28.9%, and 3 rectum-6.7%). The degree of maximum tumor vascularization was determined using the highest percentage of colored pixels obtained in the histogram- maximum color pixels density (MCPD). The hepatic Doppler perfusion index (HDPI) was also calculated. The presence and development of liver metastases was evaluated by ultrasonography and computed tomography. The patients were monitored for a period of 18 months. The results of each method in detecting and predicting the development of liver metastases were compared. **Results:** MCPD and HDPI had fairly similar results ($p>0.05$) in establishing the positive and negative predicting values for the entire group of patients with liver metastasis (55.9% compared to 66.7%, $p>0.05$, and 53.3%, compared to 54.6%, $p>0.05$) and the group that developed liver metastases during follow-up (80.0% compared to 90.0%, $p>0.05$, and 61.5%, compared to 75.0%, $p>0.05$). When comparing MCPD and HDPI for the group of patients who had or developed metastases, MCPD had an equal sensitivity (86.4%, compared to 90.9%, $p>0.05$), a higher specificity (65.0% compared to 46.5%, $p<0.05$), but a lower accuracy (60.0% compared to 73.3%, $p<0.05$). In detecting patients who developed metastases during the 18 months follow-up, MCPD had a superior sensitivity (85.7% compared to 64.3%, $p<0.05$), a lower specificity (66.7% compared to 88.9%, $p<0.05$) and an equal accuracy (78.3% vs 73.9%, $p>0.05$.). **Conclusions:** The calculation of MCPD using color histograms can be a simple and quick method in the evaluation and prognosis of patients with digestive tract adenocarcinoma.

Rezumat

Obiective: Scopul studiului este stabilirea corelației între gradul de vascularizație determinat prin power Doppler al adenocarcinoamelor (ACC) de tub digestiv și prognosticul acestor pacienți. **Material și metodă:** Au fost examinați ecografic prospectiv 45 pacienți cu diagnosticul de ACC de tub digestiv: 16 gastrice (35,6%), 6 cec și colon ascendent (13,3%), 2 colon transvers (4,4%), 5 colon descendent (11,1%), 13 colon sigmoid (28,9%) și 3 rect (6,7%). S-a determinat gradul de maximă vascularizație tumorală prin utilizarea procentului cel mai mare de pixeli colorați obținut la histogramă densitatea maximă a pixelilor colorați (MCPD). S-a calculat indicele Doppler de perfuzie hepatică (HDPI), care reprezintă raportul dintre debitul arterial hepatic și debitul total hepatic. Prin ecografie si computer tomografie s-au urmarit prezenta metastazeor hepatice. Pacienții au fost monitorizați timp de 18 luni. S-au comparat performanțele fiecarei metode în detectarea și predictibilitatea apariției metastazelor hepatice. **Rezultate:** MCPD și HDPI au avut performațe relativ egale ($p>0,05$) în ceea ce privește valoarea predictivă pozitivă (VPP) și negativă (VPN) atât pentru întreg lotul de pacienți cu metastaze hepatice (55,9% față de 66,7%, $p>0,05$, respectiv 53,3%, față de 54,6%, $p>0,05$), cât și pentru pacienții care au dezvoltat metastaze în perioada de monitorizare (80,0% față de 90,0%, $p>0,05$, respectiv 61,5%, față de 75,0%, $p>0,05$). Comparând MCPD cu IDPH la întreg lotul de pacienți care au avut sau au dezvoltat metastaze hepatice, am constat că MCPD a avut o sensibilitate egală (86,4%, față de 90,9%, $p>0,05$), o specificitate superioară (65,0% față de 46,5%, $p<0,05$), dar o acurateţe mai mică (60,0% față de 73,3%, $p<0,05$). În detectarea pacienților care au dezvoltat metastaze în cele 18 luni de urmărire, MCPD a avut o sensibilitate mai mare (85,7% față de 64,3%, $p<0,05$), o specificitate mai mică (66, 7% vs 88, 9%, $p<0,05$) și acurateţe egală (78,3% vs 73,9%, $p>0,05$), **Concluzii:** Determinarea MCPD obținut cu ajutorul histogramelor color poate fi o metodă simplă și rapidă în evaluarea și prognosticul pacienților cu adenocarcinom de tub digestiv.

Received 15.12.2010 Accepted 30.04.2011
Med Ultrason
2011, Vol. 13, No 3, 207-214
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Introduction

Tumor cell invasion within the blood vessels and/or lymph vessels represents a key moment in metastases development [1,2]. Metastases are made up from groups of tumor cells released in the blood stream by the neoformation vessels. That is why it is considered that tumor an-

giogenesis is tightly related to hematogenous metastasis. A tumor is more aggressive as it contains a larger number of neoformation vessels, leading to the conclusion that tumor vascularization study may offer important information regarding the risk of recurrence and the prognosis [3].

The intensity of tumor microvascularization may be expressed by calculating microvessel density – MVD [4]. This is a direct method for appreciating the degree of tumoral activity and risk of metastasis. Because tumoral neovascularization develops through vessels with a 2-20 microns diameter, these vessels can be demonstrated only by immunohistochemistry [5] or microscopy [6] and not by Doppler ultrasonography [7] or other imaging techniques [5,7,8].

Doppler ultrasonography is capable to detect blood flow in vessels measuring more than 100 microns in diameter [7,9,10]. With the help of new technological progresses the presence of vascular signal could be demonstrated even in superficial vessels measuring at least 40 microns [11]. At this size the intratumoral vessels are actually arterioles, venules and shunts between arterioles and venules [7,9]. The more neoformation vessels there are the more arterioles and venules with a diameter over 100 microns will be present [10].

There is a relationship between the size of the group of tumor cells present in the blood stream and the number of metastases. Administering groups of 6-7 cells produce a significantly higher number of metastases than the same number of cells injected individually [12-14]. Also, the higher the number of intratumoral vessels measuring over 100 microns the more multicellular groups will be released in circulation [13,14].

There is a correlation between the tumor vascularization demonstrated by Doppler ultrasound and MVD detected by immunohistochemistry [15]. It was demonstrated that intratumoral vascular density correlates better with patient's prognosis than the counting of vessels on histology specimens [10].

From a technical point of view, the degree of tumor hypervascularity can be assessed with the help of color histograms. The proportion of colored pixels present in an ultrasonographic image was defined as the colored pixels density (CPD) [10]. The histograms determine the CPD and thus identify the degree in which the tumor is vascularized on a certain ultrasound image. The benefit of using gray scale or color scale histograms in ultrasonography was demonstrated in multiple studies, as a reproducible method with acceptable inter- and intraobserver variations [16-20].

Digestive tract tumors usually produce metastases of the liver. Many studies proved that in the presence of liver metastases the arterial hepatic flow compared with the total hepatic flow is increased [21-23]. The hepatic Doppler perfusion index (HDPI) is defined as a ratio between the hepatic arterial flow (in the main hepatic artery) and the total hepatic flow (the sum of hepatic arterial flow and hepatic venous flow in the portal vein). In normal situations the HDPI does not exceed 30%, but it raises much over this value in the presence of liver metastases [21,22].

The purpose of this paper is to study the correlation between digestive tract adenocarcinoma vascularization quantified through histograms and the presence of liver metastases as well as to evaluate the risk of liver metastasis and establish the prognosis for these patients.

Material and method

The study was conducted in accordance with the guidelines proposed in The Declaration of Helsinki and was approved by the Local University and Hospital Ethics Committee. All patients gave their informed consent.

A number of 45 patients (31 males and 14 females), between 35 and 93 years old, with previous histological diagnosis of digestive tract adenocarcinoma (16 stomach-35.6%, 6 cecum and ascending colon-13.3%, 2 transverse colon - 4.4%, 5 descending colon - 11.1%, 13 sigmoid colon - 28.9%, and 3 rectum - 6.7%) were included in the study. The patients did not receive chemotherapy and did not undergo radiation. Ultrasound of the tumor was performed before surgery with a Voluson 730 PRO ultrasound machine, equipped with 4 to 12 MHz transducers, the chosen frequency depending on the depth of the tumor. The examination was performed on fasting patients, in supine position. In order to detect liver metastases abdominal ultrasound and CT scans were acquired, using the classical diagnosis criteria [24-28]. Patients were monitored with ultrasonography and computed tomography for liver metastases for a period of 18 months. The CT examination was considered the gold standard in the diagnosis of liver metastases.

Calculation of maximum color pixels density in digestive tract tumors. Using the software of the ultrasound machine, the CPD was calculated on 3 distinct ultrasonographic images, subjectively appreciated as intensely vascularized. **The maximum color pixeles density (MCPD)** was defined as the highest value of the CPD. The sample for the histogram was chosen in such a way that it was tangent to the tumor margins on that specific image (fig 1, fig 2). The settings of the ultrasound machine were changed in a way that resulted in a maximum sensitivity of the power Doppler function for the examined lesion. 1st degree vascularity was defined for val-

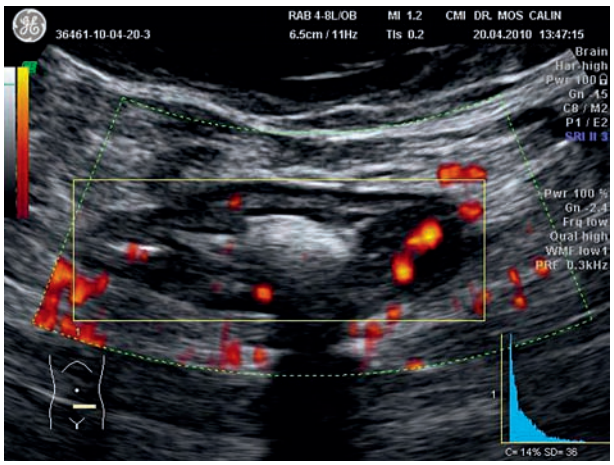

Fig 1. Transverse view of the mass. The histogram sample must be tangent to the margins of the lesion and contain the whole tumor.

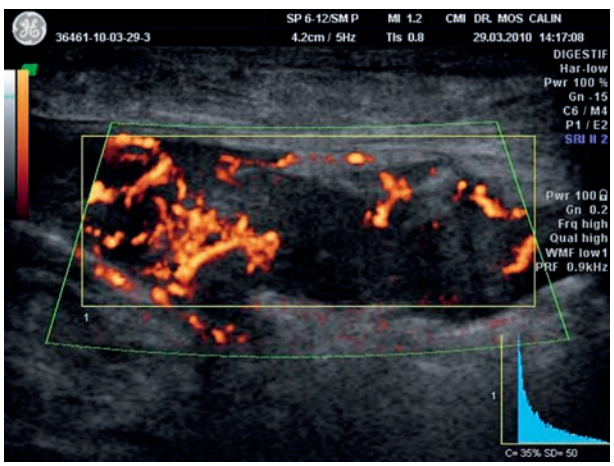

Fig 2. Setting the histogram sample on the longitudinal view of the tumor. There is a completely anarchical hypervascularity with several vascular poles. MCPD=35%.

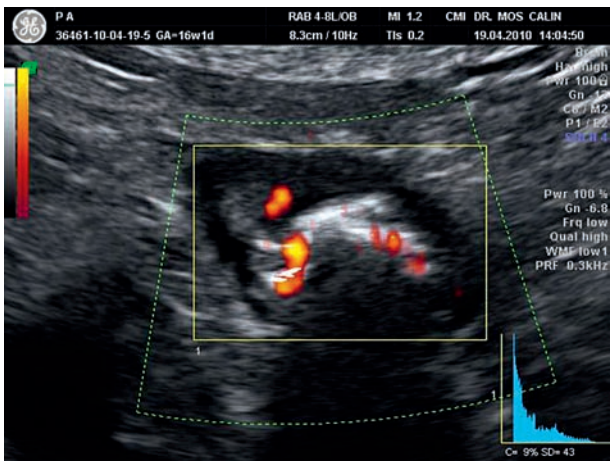

Fig 3. 1st degree vascularity. A low value of the MCPD (9%) is obtained on the histogram.

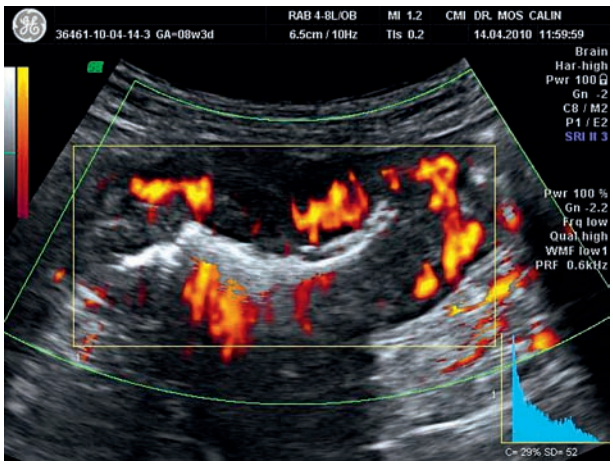

Fig 4. 2nd degree vascularity. The histogram reveals an elevated MCPD (29%).

ues of the MCPD lower than 25% (fig 3) and 2nd degree vascularity for values equal or higher than 25% (fig 4).

Calculation of the hepatic Doppler perfusion index (HDPI). The ultrasonography examination was performed in expiratory apnea. P**ortal flow** was determined using a subcostal or intercostal approach in a longitudinal view of the portal vein trunk, anterior to the inferior vena cava. The Doppler sample was placed lower than the origin of the left and right portal branches. The **flow in the main hepatic artery** was evaluated using a longitudinal view of the artery after it emerges from the common hepatic artery, anterior to the portal trunk. In both vessels a time average mean velocity (TAV mean) was obtained in the Doppler curve and the **area of section** was calculated. The **flow volume/min** was calculated by multiplying the TAV mean, the area of vascular section and the heart rate. The HDPI was considered normal if it was lower or equal to 30%.

Patients were monitored with ultrasonography and CT for the detection of liver metastases for 18 months.

Statistical analysis. The statistical analysis was performed with EPIINFO, application of CDC - Center of Disease Control and Prevention of Atlanta and World Health Organization for medical statistics. We calculated average and standard deviation, frequency intervals and we used the Student (t test) method and c^2 as tests of significance. P value <0.05 was considered statistically significant. We calculated the detectability index for comparison of predictive capacity between the two methods.

Results

At the first examination 22 out of the 45 patients (48.89%) had liver metastases. The CT scan showed the presence of metastases in 21 patients (95.45%) and

the ultrasonography in 17 (77.27%). At the end of the follow-up period liver metastases were identified in 14 more cases with CT (the ultrasound was able to identify 11 of these patients – 78.57%). MCPD obtained in the 45 patients using histograms was between 5% and 76%. The distribution of patients based on the values of the HDPI and MCPD is illustrated in table I.

The distribution and the evolution of the patients with or without liver metastases in relationship with the MCPD values are presented in figure 5.

HDPI and MCPD measures of performance in detection and prediction of liver metastases development are presented in table II.

If the entire group of patients with liver metastases is considered, MCPD had a superior specificity compared with the HDPI (65.0% versus 46.5%, $p<0.05$), but a lower accuracy (60.0% compared with 73.3%, $p<0.05$). For this group of patients the differences of sensitivity, positive predictive value and negative predictive value between the two methods were not statistically significant ($p>0.05$).

Comparing the patients without liver metastases at diagnosis, but who developed them during the follow-up period, a higher sensitivity of the MCPD compared with the HDPI was noted (85.7% compared with 64.3%,

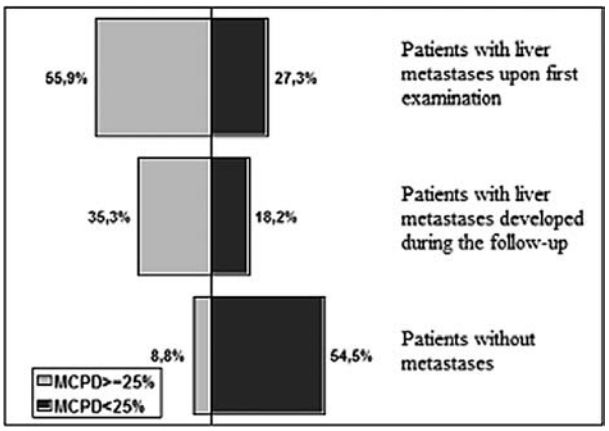

Fig 5. The distribution and evolution of the patients in relationship with maximum color pixels density (MCPD) values.

Table I. Distribution of the patients based on the values of the hepatic Doppler perfusion index and maximum color pixeles density

	Number of patients	HDPI		MCPD	
		>30%	≤30%	≥ 25%	< 25%
Patients with liver metastases at first examination	22 (48.9%)	20 (90.9%)	2 (9.1%)	19 (86.4%)	3 (13.6%)
Patients with liver metastases developed during the follow-up	14 (31.1%)	9 (64.3%)	5 (35.7%)	12 (85.7%)	2 (14.3%)
Ppatients without metastases	9 (20.0%)	1 (11.1%)	8 (88.9%)	3 (33.37%)	6 (66.7%)
Total number of patients	45 (100%)	30 (66.7%)	15 (33.3%)	34 (75.6%)	11 (24.4%)

HDPI- hepatic Doppler perfusion inde, MCPD- maximum color pixeles density

Table II. Comparison of performance of hepatic Doppler perfusion index and maximum color pixeles density in patients with liver metastasis

	Patients with metastases at first examination (22 patients)			Patients with metastases developed during the monitoring period (14 patients)		
	HDPI	MCPD	p	HDPI	MCPD	p
Sensitivity (%)	90.90	86.37	>0.05	64.29	85.71	0.025
Specificity (%)	46.48	65.00	0.037	88.89	66.67	0.034
Accuracy (%)	73.33	60.00	0.048	73.91	78.26	>0.05
PPV (%)	66.67	55.88	>0.05	90.00	80.00	>0.05
NPV (%)	54.55	53.33	>0.05	75.00	61.54	>0.05

HDPI-hepatic Doppler perfusion index, MCPD-maximum color pixeles density PPV-positive predictive value, NPV-negative predictive value

p<0.05), while the specificity of MCPD was lower than that of the HDPI (66.7% compared with 88.8%, p<0.05). In the patients without metastasis during the monitoring period there were no statistically significant differences regarding accuracy and positive and negative predictive values (p>0.05).

By calculating the detectability index it was determined that HDPI is able to diagnose liver metastases 1.22 times more accurately than MCPD with a 25% threshold (p>0.05). On the other hand MCPD predicts the development of liver metastases during the 18 months follow-up 1.06 times more accurately than HDPI, with a 25% threshold.

The value of MCPD was 10.66 +/- 3.42 for ~~the~~ patients who did not develop metastases during the study and 42.32 +/- 11.31 for those who did (p<0.001).

The survival rate after 18 months was 70.59% for the patients with ~~a~~ MCPD ≥ 25% (for ~~a~~ MCPD > 60% the survival rate was 16.67%). For ~~a~~ MCPD < 25% the survival rate at 18 months was 90.91%.

Discussions

Alterations of the hepatic flow in tumors were initially observed using dynamic scintigraphy. In 1983 Parkin et al [29] proved that when malignant tumors are present the arterial hepatic flow is elevated because tumors have a predominantly arterial vascularity. Parkin also proposed the use of the *hepatic perfusion index (HPI)*, which is increased in patients with liver metastases [29]. Additionally, in the presence of metastases, it appears that there is a vasoactive circulatory agent that reduces the portal flow [30,31]. In 1985, Levanson et al [32] showed that HPI can be useful in the diagnosis of occult metastases developed from colorectal cancer. In 1991, Leen et al [33] defined *HDPI* and proposed Doppler ultrasonography for the quantification of liver vascularity alterations, demonstrating its usefulness in subsequent studies [21,22,34-36]. In normal conditions HDPI does not exceed 30%. In the presence of liver metastases HDPI can increase considerably over 30%, reaching values as high as 80% [21,22].

Grey-scale or color histograms (plane or three-dimensional) were used in the study of the liver [37,38], thyroid [39-42], oral cavity and salivary glands [43,44], urinary bladder [45] but mostly in obstetrics and gynecology [18, 46-50]. According to the vast majority of the studies, using histograms proved to be useful and reproducible in the quantification of vascularity. In the particular case of digestive tract tumors histograms have been used less often [10]. The authors considered that the vascularity of digestive tract tumors can also be characterized by using color histograms more objectively than by operator subjective evaluation.

In this study MCPD was given by the highest value of the CPD not by the mean of the obtained values. It was assumed that the tumor area with the most increased vascularity most faithfully represents tumor aggressiveness. The lower values obtained on the histogram on certain views does not characterize the tendency to metastasis of the tumor. Hypovascular areas may be caused by the presence of nonvascular spaces created by intratumoral necrosis, which does not mean that the tumor is not aggressive.

For the calculation of MCPD with color histograms it was considered that the standard settings of the ultrasound machine are not the best option due to the differences in tumor depth. The differences of weight between patients and of tumor depth made it necessary to use transducers with adapted frequencies (4-12 MHz). The introduction of small peritumoral areas in the histogram samples did not alter the results due to peritumoral hypervascularity.

Comparing the study groups the conclusion was that there are no statistically significant differences between the two methods (MCPD and HDPI) as far as the positive and negative predictive values and sensitivity are concerned (p>0.05). The sensitivity for the group of patients with metastases at the beginning of the study and the patients who developed metastasis during follow-up were equal (p>0.05). There were no statistically significant differences in the accuracy of the two methods (p>0.05) in identifying patients without metastases at the beginning of the study, but who subsequently developed them.

For the entire group of patients with liver metastases MCPD has a superior specificity than HDPI (p<0.05), but a lower accuracy (p<0.05). In the case of patients who did not initially have metastases, but developed them in the 18 months follow-up, it was noted that the sensitivity of the MCPD was higher (p<0.05), but the specificity proved to be lower (p<0.05).

Even though the differences were not statistically significant, in this study the MCPD was inferior to HDPI in detecting existing metastases, but was superior to HDPI in predicting their future development. The analysis of the images with the highest proportion of colored pixels led to the observation that in these areas the number of tumoral vascular poles was also higher. In extremely hypervascularized tumors, with MCPD > 60%, short-term life expectancy was reduced (survival rate at 18 months was only 16.66%).

A statistically significant difference (p< 0.001) was noticed between the mean values of MCPD in patients who did not develop metastases compared with those

who did develop them in the follow-up period. This finding suggests that MCPD may be used in the assessment and prognosis of the patients with digestive tract adenocarcinoma.

MCPD is considered a much simpler and quicker method than HDPI in predicting metastases development. While HDPI calculation is highly time-consuming and often technically difficult to realize (hepatic artery anomalies, obese patients, aerocoly, difficulties maintaing the expiratory apnea), MCPD calculation ~~it~~ is easy, it takes a few minutes at the most and can be determined every time a digestive tract mass is visualized on the ultrasonographic exam. MCPD can also be correlated with other characteristics of the tumor vascularity, like identifying the number of tumoral vascular poles, and thus contribute to increase method accuracy.

The main limit of this study is represented by the fact that the investigated tumors were found at different depths, situation that required the use of multiple frequencies. A mass appears more hypervascular if it is situated closer to the transducer and less vascularized if it is located further from it. Also the particular sensitivities of the Doppler functions for different ultrasound machines did not allow the use of the same reference value of the MCPD for all the machines. The reduced number of patients included in the study did not permit a comparison between the different sites of the masses. Another important limit of the study is the lack of intra- and inter-observer variation assessment.

Conclusions

The calculation of the MCPD in digestive tract masses is an indirect method used to evaluate the presence of liver metastases. The method has good results, comparable with direct, imaging visualization of the tumor. Furthermore, it contributes to the prediction of future development of liver metastases. In conclusion, discovering the degree of tumor vascularity, with the help of color histograms can be a simple, quick method of evaluation and prognosis for the patients with digestive tract adenocarcinoma.

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